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THEORY OF CHROMATOGRAPHY OF RIGID ROD-LIKE MACROMOLECULES ON HYDROXYAPATITE COLUMNS

VI. ANALYTICAL CALCULATION OF CHROMATOGRAMS*

TSUTOMU KAWASAKI

Laboratoire de Génétique Moléculaire, Institut de Biologie Moléculaire, Faculté des Sciences, 75005-Paris (France)

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SUMMARY

The evolution of rod-like macromolecules on hydroxyapatite columns has been treated analytically and general shapes of the theoretical chromatograms have been calculated for the cases of (1) a single molecular species, (2) a mixture of several molecular species and (3) a mixture of a large number of species distributed continuously.

INTRODUCTION

In Parts I-V of this series¹⁻⁵, we developed a theory of chromatography of rigid rod-like macromolecules taking into account the fact that there are mutual interactions among molecules adsorbed on the surface of hydroxyapatite (HA). The dynamic part of the chromatographic process has been studied by dividing the column into a finite number of sections, which permits, however, only numerical calculations of chromatograms to be made. In this paper, we treat the dynamic part analytically by solving the differential equation that expresses the variation of the concentration of the macromolecules on the column. The study is limited to the actual case when there are repulsive forces among adsorbed macromolecules^{1,2}. We have also restricted the study to the case when the slope of the activity gradient of competing ions is linear.

THEORETICAL

The case of a single molecular species

In Parts I and II^{1,2} we mentioned that the chromatogram, as a function of the activity of competing ions, is generally independent of the slope of the gradient of the activity (see also Discussion), so that the conclusion drawn by assuming that the slope is very small is valid even when the slope is large. When the slope is very small, the width of the chromatogram is much larger than the column length, *i.e.*, the total interstitial volume of the column is much smaller than the total volume of the eluent in which macromolecules are involved. It can be said, therefore, that the

* Please note that in Part III of this series (*J. Chromatogr.*, 82 (1973) 219) eqns. 8-13 should be deleted, since these include incorrect expressions. As all calculations were carried out directly by using the Monte Carlo method, and not by using these expressions, eqns. 8-13 are not necessary.

number of macromolecules that exist in the interstitial liquid of the column at time t is practically equal to the loss of macromolecules from the column when a solution with the same volume as that of the column interstice is eluted and that this is also equal to the loss of adsorbed molecules from the crystal surfaces. Now, we can have the following differential equation:

$$-\frac{d\chi}{dV} = \frac{B}{1-B} \chi \quad (1)$$

in which χ is the parameter defined by eqn 49 in Part I¹. Here we can consider that χ simply expresses the relative amount of macromolecules adsorbed on the crystal surfaces in the whole column, B is a measure of the proportion of macromolecules existing in the solution (see eqn. 27 in Part I) and V is the elution volume. As the activity of competing ions y (see eqn. 43 in Part I) is proportional to V , choosing the proportionality constant as unity*, eqn. 1 can be rewritten as

$$-\frac{d\chi}{dy} = \frac{B}{1-B} \chi \quad (1a)$$

The right-hand side of eqn. 1 or 1a expresses the number of molecules in the interstitial liquid of the column and the chromatogram (denoted by f) as a function of V or y is given by

$$f(y) = \frac{B}{1-B} \chi \quad (2)$$

The parameter B can be expressed as a function of y and χ as

$$\left. \begin{array}{l} B=0 \quad (\chi < \chi'(y)) \\ B=1 \quad (\chi > \chi'(y)) \end{array} \right\} \quad (3)$$

in which $\chi'(y)$ is the capacity (expressed as a relative value) of the adsorbent when the activity of the competing ions is equal to y . Eqn. 3 is self-evident from the physical meaning of χ' , which can be expressed as eqn. 56 in Part I:

$$\left. \begin{array}{l} \chi'(y) = 1 \quad (y < 1 - \Xi) \\ \chi'(y) = \left\{ \frac{1}{\Xi} (1 - y) \right\}^2 \quad (1 - \Xi \leq y < 1) \\ \chi'(y) = 0 \quad (y \geq 1) \end{array} \right\} \quad (4)$$

In eqn. 4, Ξ is the parameter concerning the mutual interactions of molecules on the crystal surfaces and has a positive value. If there are no such interactions, $\Xi = 0$.

The value of y is usually virtually zero when the sample is loaded on the column and the sample remains on the crystal surfaces during the rinsing process before the gradient begins^{1,2}. Now, denoting the number of macromolecules loaded by χ^* ,

* It is easy to understand that the chromatogram as a function of y is independent of the proportionality constant as far as the relative value of f is concerned (see below).

the shape of the chromatogram as a function of y can be calculated by solving eqn. 1a for χ under the initial conditions

$$y=0 \quad (5)$$

and

$$\chi=\chi^* \quad (6)$$

and by substituting the solution into eqn. 2. It should be noted that the value of χ^* is unity if the column is initially saturated with macromolecules; it is only when $\bar{\Xi} \leq 1$ that the state of saturation can be reached. A value of $\chi^* < 1$ can be caused either by the fact that the maximum possible number of molecules is loaded but that the saturation state cannot be realized as $\bar{\Xi}$ is greater than unity (see Fig. 1a and 1b in Part I) or by the fact that the number of molecules loaded is less than the maximum possible number. It should be also noted that the value of χ^* must always be less than or equal to $1/\bar{\Xi}^2$ (see Part I).

Now, solving eqn. 1a, when $\chi^* < \chi'(y)$ or when

$$0 \leq y < 1 - \bar{\Xi} \sqrt{\chi^*} \quad (7)$$

it is evident that $B=0$ (see eqn. 3) and from eqns. 1a and 2 we have

$$\chi=\chi^* \quad (8)$$

and

$$f(y)=0 \quad (9)$$

When $\chi^* \geq \chi'(y)$, if

$$1 - \bar{\Xi} \sqrt{\chi^*} \leq y < 1 \quad (10)$$

from the physical meaning of $\chi'(y)$, then

$$\chi=\chi'(y) = \left\{ \frac{1}{\bar{\Xi}} (1-y) \right\}^2 \quad (11)$$

where the second equality in eqn. 11 is due to the fact that, for a range of y given by $1 - \bar{\Xi} \leq 1 - \bar{\Xi} \sqrt{\chi^*} \leq y < 1$, the second expression in eqn. 4 holds. Hence, from eqns. 1a and 2,

$$f(y) = - \frac{d\chi}{dy} = \frac{2}{\bar{\Xi}^2} (1-y) \quad (12)$$

is obtained. Finally, if we substitute $y=1$ into eqns. 11 and 12, then $\chi=0$ and $f=0$, so that when

$$y \geq 1 \quad (13)$$

then

$$\chi=0 \quad (14)$$

and

$$f(y)=0 \quad (15)$$

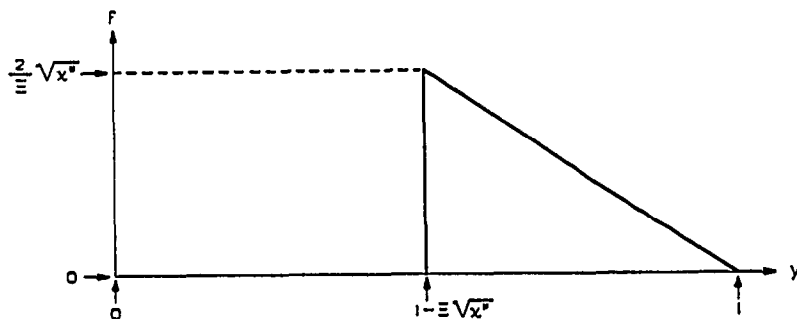


Fig. 1. Illustration of the general shape of the chromatogram in the case of a single molecular species. It was assumed that

$$\frac{2}{3}\sqrt{\chi^*} = 0.5 \text{ and } w = 1.$$

Fig. 1 illustrates the general shape of the chromatogram. As far as the relative value of f is concerned, the shape of the chromatogram is governed only by a factor $\frac{2}{3}\sqrt{\chi^*}$, so that the chromatogram calculated by replacing χ^* with χ^*/α , where α is a constant, is identical with that obtained when the column is α times longer, when the number of macromolecules loaded is α times smaller or when the value of $\frac{2}{3}$ is $\sqrt{\alpha}$ times smaller.

The case of several molecular species

Following the same procedure as for a single molecular species, we have the following simultaneous differential equations which show the evolution of molecular species 1, ..., ρ' , ..., ρ on the column:

$$-\frac{d\chi_{(\rho')}}{dV} = \frac{B_{(\rho')}}{1 - B_{(\rho')}} \chi_{(\rho')} \quad (16)$$

($\rho' = 1, 2, \dots, \rho$), or

$$-\frac{d\chi_{(\rho')}}{dy} = \frac{B_{(\rho')}}{1 - B_{(\rho')}} \chi_{(\rho')} \quad (16a)$$

($\rho' = 1, 2, \dots, \rho$), in which we can consider simply that $\chi_{(\rho')}$ expresses the relative amount in weight of species ρ' adsorbed on the crystal surfaces in the column, as each molecule has a very elongated shape, and $B_{(\rho')}$ is a measure of the proportion of species ρ' that exists in the solution (see eqn. 38 in Part II²). The right-hand side of eqn. 16 or 16a expresses the amount of species ρ' in the interstitial liquid of the column, and the contribution of species ρ' (denoted by $f_{(\rho')}$) to the total chromatogram is given by

$$f_{(\rho')}(y) = \frac{B_{(\rho')}}{1 - B_{(\rho')}} \chi_{(\rho')} \quad (17)$$

Now, assigning the values 1, 2, ..., ρ to ρ' , following the decrease in the preference of the adsorption of a particular molecular species when it is being eluted,

we have the relationship $\rho' < \rho''$ if $w_{(\rho')} > w_{(\rho'')}$ or if $w_{(\rho')} = w_{(\rho'')}$ and $x'_{(\rho')} > x'_{(\rho'')}$, which has been shown in Part V⁵. We have also shown, in Part II², that the state of a molecular species ρ' on the crystal surface is influenced only by species 1, 2, ..., $\rho' - 1$ and by the other molecules of the same species, ρ' , so that $B_{(\rho')}$ can be expressed as a function of y and $\chi_{(1)}, \chi_{(2)}, \dots, \chi_{(\rho')}$, as

$$\left. \begin{aligned} B_{(\rho')} &= 0 \left(\sum_{\rho''=1}^{\rho'} \chi_{(\rho'')} < \chi'_{(\rho')}(y, w_{(\rho')}) \right) \\ B_{(\rho')} &= 1 \left(\sum_{\rho''=1}^{\rho'} \chi_{(\rho'')} > \chi'_{(\rho')}(y, w_{(\rho')}) \right) \end{aligned} \right\} \quad (18)$$

In eqn. 18, $\chi'_{(\rho')}(y, w_{(\rho)})$ is the maximum possible value of χ which can be realized by all of species 1, 2, ..., ρ' when the activity of competing ions is y , and can be expressed as eqn. 69 in Part II:

$$\left. \begin{aligned} \chi'_{(\rho')}(y, w_{(\rho')}) &= 1 & (y < w_{(\rho')} - \Xi) \\ \chi'_{(\rho')}(y, w_{(\rho')}) &= \left\{ \frac{1}{\Xi} (w_{(\rho')} - y) \right\}^2 & (w_{(\rho')} - \Xi \leq y < w_{(\rho')}) \\ \chi'_{(\rho')}(y, w_{(\rho')}) &= 0 & (y \geq w_{(\rho')}) \end{aligned} \right\} \quad (19)$$

Now, eqn. 16a must be solved for $\chi_{(1)}, \chi_{(2)}, \dots, \chi_{(\rho)}$ under the initial conditions given by eqn. 5 and by the relationships

$$\chi_{(\rho')} = \chi^*_{(\rho')} \quad (20)$$

($\rho' = 1, 2, \dots, \rho$) and functions $f_{(1)}(y), f_{(2)}(y), \dots, f_{(\rho)}(y)$ must be calculated by substituting the solutions into eqn. 17. It should be noted that the value of

$$\sum_{\rho''=1}^{\rho} \chi^*_{(\rho'')}$$

is unity if the column is initially saturated with macromolecules. It is evident that when

$$\sum_{\rho''=1}^{\rho'} \chi^*_{(\rho'')} < \chi'_{(\rho')}(y, w_{(\rho')}) \quad (21)$$

or when

$$0 \leq y < w_{(\rho')} - \Xi \sqrt{\sum_{\rho''=1}^{\rho'} \chi^*_{(\rho'')}} \quad (22)$$

$B_{(\rho')} = 0$ (see eqn. 18) and from eqns. 16a and 17 we have

$$\chi_{(\rho')} = \chi^*_{(\rho')} \quad (23)$$

and

$$f_{(\rho')}(y) = 0 \quad (24)$$

When

$$\sum_{\rho''=1}^{\rho'-1} \chi^*_{(\rho'')} < \chi'_{(\rho')}(y, w_{(\rho')}) \leq \sum_{\rho''=1}^{\rho'} \chi^*_{(\rho'')} \quad (25)$$

or when

$$w_{(\rho')} - \Xi \sqrt{\sum_{\rho''=1}^{\rho'} \chi^*_{(\rho'')}} \leq y < w_{(\rho')} - \Xi \sqrt{\sum_{\rho''=1}^{\rho'-1} \chi^*_{(\rho'')}} \quad (26)$$

from the physical meaning of $\chi'_{(\rho')}(y, w_{(\rho')})$, we have

$$\sum_{\rho''=1}^{\rho'} \chi_{(\rho'')} = \chi'_{(\rho')}(y, w_{(\rho')}) = \left\{ \frac{1}{\Xi} (w_{(\rho')} - y) \right\}^2 \quad (27)$$

or

$$\chi_{(\rho')} = \left\{ \frac{1}{\Xi} (w_{(\rho')} - y) \right\}^2 - \sum_{\rho''=1}^{\rho'-1} \chi_{(\rho'')} \quad (27a)$$

where the sum

$$\sum_{\rho''=1}^0 \chi^*_{(\rho'')},$$

which appears when $\rho' = 1$, is zero and where the second equality in eqn. 27 is due to the fact that, for a range of y given by

$$w_{(\rho')} - \Xi \leq w_{(\rho')} - \Xi \sqrt{\sum_{\rho''=1}^{\rho'} \chi^*_{(\rho'')}} \leq y < w_{(\rho')} - \Xi \sqrt{\sum_{\rho''=1}^{\rho'-1} \chi^*_{(\rho'')}} < w_{(\rho')}$$

the second expression of eqn. 19 holds. On the other hand, we can have

$$\sum_{\rho''=1}^{\rho'-1} \chi_{(\rho'')} = \sum_{\rho''=1}^{\rho'-1} \chi^*_{(\rho'')} \quad (28)$$

because by representing one of $1, 2, \dots, \rho' - 1$ by ρ''' and using the left-hand inequality in eqn. 25 in this paper and eqn. 72 in Part II, which is valid for any type of mixtures, *i.e.*,

$$\chi'_{(\rho^*)}(y, w_{(\rho^*)}) \geq \chi'_{(\rho^{**})}(y, w_{(\rho^{**})}) \quad (29)$$

($\rho^* < \rho^{**}$), we obtain

$$\sum_{\rho''=1}^{\rho'''} \chi^*_{(\rho'')} \leq \sum_{\rho''=1}^{\rho'-1} \chi^*_{(\rho'')} < \chi'_{(\rho^*)}(y, w_{(\rho^*)}) \leq \chi'_{(\rho^{**})}(y, w_{(\rho^{**})}) \quad (30)$$

The relationship between the extreme left-hand term and the extreme right-hand term in eqn. 30 is the same as eqn. 21. Hence we have

$$\chi_{(\rho^*)} = \left\{ \frac{1}{\Xi} (w_{(\rho^*)} - y) \right\}^2 - \sum_{\rho''=1}^{\rho'-1} \chi^*_{(\rho'')} \quad (31)$$

and

$$f_{(\rho')} (y) = -\frac{d\chi_{(\rho')}}{dy} = \frac{2}{\Xi^2} (w_{(\rho')} - y) \quad (32)$$

where it should be noted that relationships

$$\chi_{(\rho')} = 0 \text{ and } f_{(\rho')} = \frac{2}{\Xi^2} \sqrt{\sum_{\rho''=1}^{\rho'-1} \chi^*_{(\rho'')}}$$

can be obtained if the relationship

$$y = w_{(\rho')} - \Xi \sqrt{\sum_{\rho''=1}^{\rho'-1} \chi^*_{(\rho'')}}$$

is substituted into eqns. 31 and 32, respectively. Hence, when

$$y \geq w_{(\rho')} - \Xi \sqrt{\sum_{\rho''=1}^{\rho'-1} \chi^*_{(\rho'')}} \quad (33)$$

then

$$\chi_{(\rho')} = 0 \quad (34)$$

and

$$f_{(\rho')} = 0 \quad (35)$$

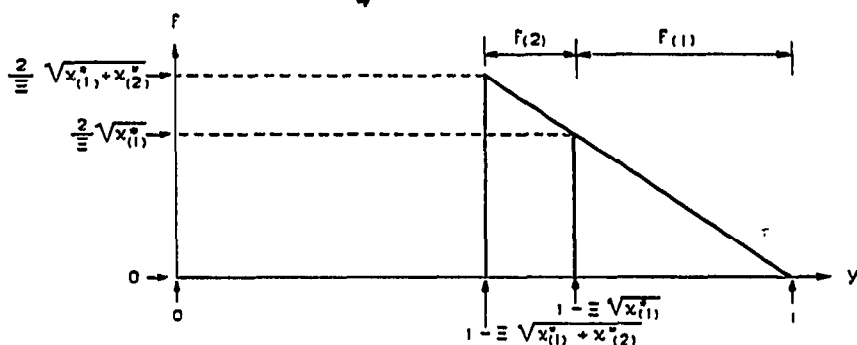


Fig. 2. As Fig. 1 for the case of a mixture of two molecular species, 1 and 2, with the same adsorption energy per unit molecular length but with different lengths. It was assumed that

$$\Xi \sqrt{\chi^*_{(1)} + \chi^*_{(2)}} = 0.5, \chi^*_{(1)} = \chi^*_{(2)} = \chi^*/2, w_{(1)} = w_{(2)} = 1 \text{ and } x'_{(1)} > x'_{(2)}.$$

Figs. 2 and 3 illustrate the general shapes of the chromatograms of mixtures of two molecular species, 1 and 2, where it has been assumed that $\chi^*_{(1)} = \chi^*_{(2)} = \chi^*/2$, χ^* being the initial value of χ in Fig. 1. Fig. 2 is the case when $1 = w_{(1)} = w_{(2)}$ and $x'_{(1)} > x'_{(2)}$, and Fig. 3 is the case when $1 = w_{(1)} > w_{(2)}$. It can be seen that when

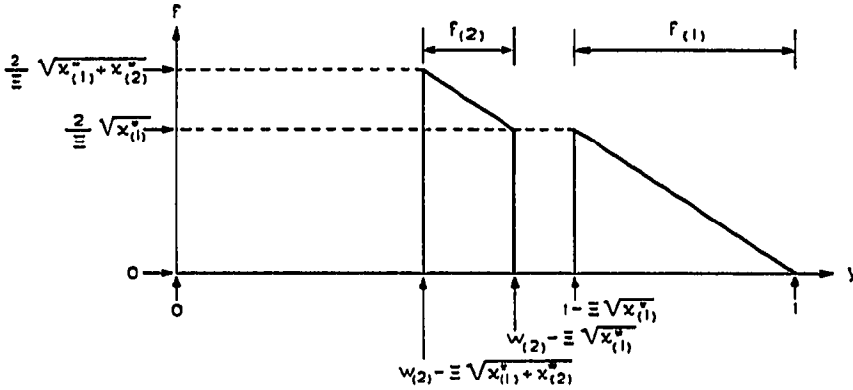


Fig. 3. As Fig. 2 when the two species, 1 and 2, have different adsorption energies per unit molecular length. It was assumed that

$$w_{(1)} = 1 \text{ and } w_{(2)} = 0.9.$$

$1 = w_{(1)} = w_{(2)}$, *i.e.*, when the charge per unit length is the same for both species, the total chromatogram is identical with that obtained in the case of a single molecular species and that species 2 with the lower value of x' is displaced to the left (see Fig. 2). When $1 = w_{(1)} > w_{(2)}$ (see Fig. 3), the contribution of species 1 to the total chromatogram is the same as in the case when $1 = w_{(1)} = w_{(2)}$. The shape of the contribution of species 2 is also the same as in Fig. 2. However, this contribution results in lower values of y when $w_{(2)} < 1$ and it coincides exactly with the left-hand part of the chromatogram of a single molecular species with the relative value of ξ , w , and the initial value of χ equal to $w_{(2)}$ and χ^* , respectively (see Fig. 4). The contributions of species 1 in both Fig. 2 and Fig. 3 coincide exactly with the right-hand part of the chromatogram of a single molecular species with $w = 1$ (see Fig. 1).

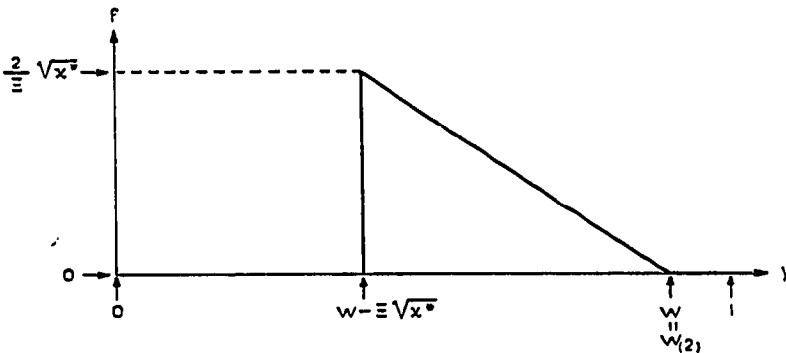


Fig. 4. As Fig. 1 when $w = 0.9$.

The case when the distribution of molecular species is continuous

It is easy to extend the theory developed above to the case of a large number

of molecular species distributed continuously. In this case, writing w^* instead of $w_{(1)}, w_{(2)}, \dots, w_{(p)}$ and calling $F(w)$ the distribution function of w , the total chromatogram $f(y)$ can be expressed as

$$f(y) = F(w) \cdot \frac{dw}{dy} \tag{36}$$

when, following eqn. 26, one has the relationship

$$y = w - \Xi \sqrt{\frac{1}{\alpha'} \int_w^\infty F(w) dw} \tag{37}$$

in which α' is a positive constant. Eqn. 37 is evident from the fact that the initial value of χ , i.e., $\chi^*(w)$, is proportional to $F(w)$. The function $f(y)$ can be calculated by solving the integral equation, eqn. 37, for w and by substituting the solution into eqn. 36. Figs. 6-10 show the results of the calculations of $f(y)$ for the distributions of molecular species shown in parts (a), (b), (c), (d) and (e), respectively, in Fig. 5. It has been assumed that $\Xi = 0.5$ and in parts (a), (b) and (c) of Figs. 6-10 the values of α' have been chosen as 1, 2 and 4, respectively, so that the column is twice and four

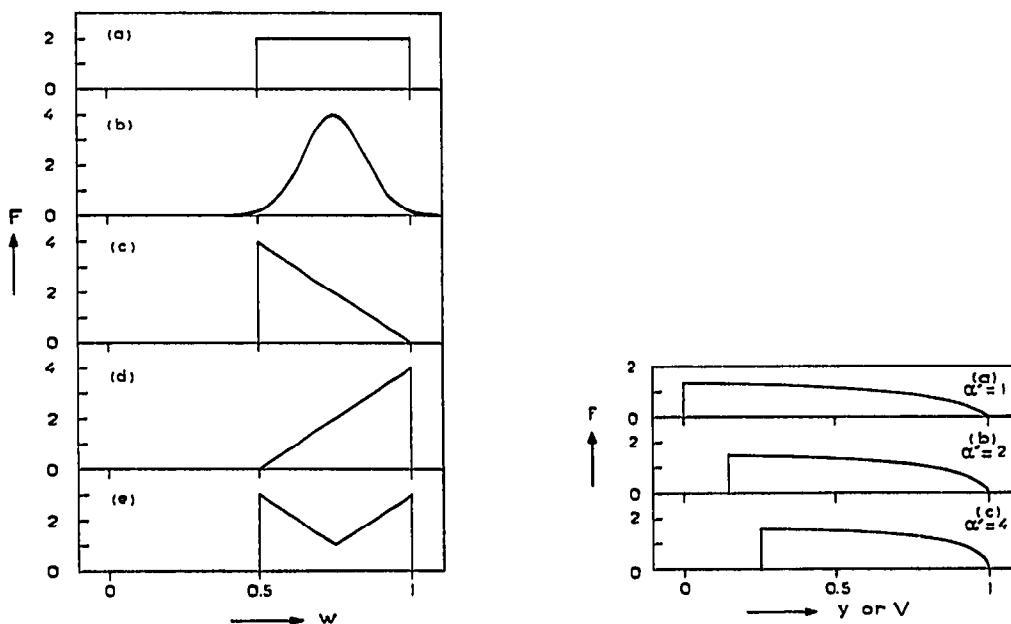


Fig. 5. Several types of continuous distributions of molecular species with different w values. The Gaussian distribution is shown in (b).

Fig. 6. Chromatograms with $\Xi = 0.5$ for the mixture as in Fig. 5a for several α' values. When $\alpha' = \infty$, the chromatogram coincides with that shown in Fig. 5a.

* This w is different from that defined in the preceding section (see Fig. 4).

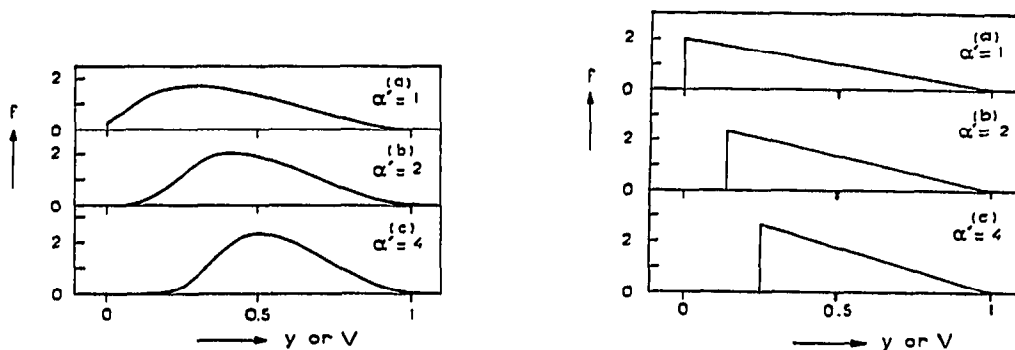


Fig. 7. As Fig. 6 for the mixture as in Fig. 5b. When $\alpha' = \infty$, the chromatogram coincides with that shown in Fig. 5b.

Fig. 8. As Fig. 6 for the mixture as in Fig. 5c. When $\alpha' = \infty$, the chromatogram coincides with that shown in Fig. 5c.

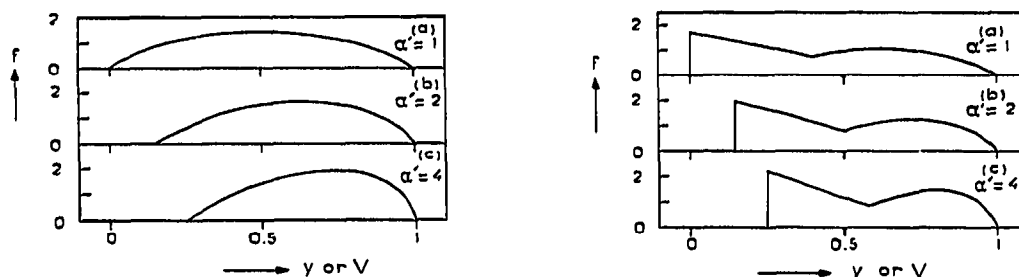


Fig. 9. As Fig. 6 for the mixture as in Fig. 5d. When $\alpha' = \infty$, the chromatogram coincides with that shown in Fig. 5d.

Fig. 10. As Fig. 6 for the mixture as in Fig. 5e. When $\alpha' = \infty$, the chromatogram coincides with that shown in Fig. 5e.

times longer in parts (b) and (c), respectively, than in part (a). It should be noted that when $\alpha' = \infty$ the chromatograms in Figs. 6–10 tend towards those in parts (a), (b), (c), (d) and (e), respectively, in Fig. 5. In cases of the distributions shown in parts (a) and (c)–(e) in Fig. 5, eqn. 37 can be solved analytically. In the case of the Gaussian distribution shown in part (b) of Fig. 5, however, it cannot be solved analytically and the calculation of the chromatograms was carried out assuming that molecules are the assembly of 500 species with differences in $w_{(p')}$ equal to 0.002 and its multiples. For practical purposes, we also divided the value of the activity, y , into elementary steps each of 0.05 unit and estimated the probabilities that molecules appear in these elements. In Fig. 7a, the chromatogram begins abruptly when $y=0$ because some molecules cannot be retained on the column when they are loaded as, in the case of a Gaussian distribution, the possible values of w extend to $-\infty$; only the molecules that can be retained appear in the chromatogram. Figs. 6 and 8–10 show the cases when all loaded molecules can be retained on the column.

Finally, we want to show that both eqns. 36 and 37 are valid even when the

distribution of molecular species in the mixture is discrete. In this case, denoting the distribution function of species ρ' that has a relative value of ξ equal to $w_{(\rho')}$ by $F'(w_{(\rho')})$, $F(w)$ could be expressed as a sum of Dirac δ -functions as

$$\left. \begin{aligned} F(w) &= \frac{1}{\varepsilon} F'(w_{(\rho')}) && (w_{(\rho')} - \varepsilon \leq w \leq w_{(\rho')}; \rho' = 1, 2, \dots, \rho) \\ F(w) &= 0 && (\text{in the other range of } w) \end{aligned} \right\} \quad (38)$$

where ε is a small and positive constant, and where it should be recalled that $w_{(\rho')} > w_{(\rho'')}$ if $\rho' < \rho''$. Now, when

$$w_{(\rho')} - \varepsilon \leq w \leq w_{(\rho')} \quad (39)$$

it is easy to calculate the integral term in eqn. 37 and we have

$$y = w - \Xi \sqrt{\sum_{\rho''=1}^{\rho'-1} F'(w_{(\rho'')})} + \frac{1}{\varepsilon} (w_{(\rho')} - w) F'(w_{(\rho')}) \quad (40)$$

where, in order to simplify the calculation, we have assumed that $\alpha' = 1$ and where the sum

$$\sum_{\rho''=1}^0 F'(w_{(\rho'')}),$$

which appears when $\rho' = 1$, is zero. Using eqn. 40, the possible values of y when w satisfies eqn. 39 can be estimated as

$$w_{(\rho')} - \Xi \sqrt{\sum_{\rho''=1}^{\rho'} F'(w_{(\rho'')})} - \varepsilon \leq y \leq w_{(\rho')} - \Xi \sqrt{\sum_{\rho''=1}^{\rho'-1} F'(w_{(\rho'')})} \quad (41)$$

Now, it is required to solve eqn. 40 for w , which is easy as it can be rewritten as a second-degree equation for w ; we obtain for the solution which satisfies the relationship

$$\begin{aligned} w &= w_{(\rho')} \text{ when } y = w_{(\rho')} - \Xi \sqrt{\sum_{\rho''=1}^{\rho'-1} F'(w_{(\rho'')})} \quad (\text{see eqns. 39 and 41):} \\ w &= y - \frac{\Xi^2}{2\varepsilon} F'(w_{(\rho')}) + \Xi \sqrt{\frac{\Xi^2}{4\varepsilon^2} \{F'(w_{(\rho')})\}^2 + \frac{1}{\varepsilon} (w_{(\rho')} - y) F'(w_{(\rho')}) + \sum_{\rho''=1}^{\rho'-1} F'(w_{(\rho'')})} \end{aligned} \quad (42)$$

Therefore, neglecting higher orders in ε , the derivative of w (see eqn. 36) can be calculated as

$$\frac{dw}{dy} \approx \frac{2\varepsilon}{\Xi^2} \cdot \frac{1}{F'(w_{(\rho')})} \cdot (w_{(\rho')} - y) \quad (43)$$

and from eqns. 43, 38 and 36

$$f(y) = \frac{2}{\Xi^2} (w_{(\rho')} - y) \quad (44)$$

is obtained. When

$$w_{(\rho'+1)} < w < w_{(\rho')} - \varepsilon \quad (45)$$

or when

$$w_{(\rho'+1)} - \Xi \sqrt{\sum_{\rho''=1}^{\rho'} F'(w_{(\rho'')})} < y < w_{(\rho')} - \Xi \sqrt{\sum_{\rho''=1}^{\rho'} F'(w_{(\rho'')})} - \varepsilon \quad (46)$$

it is evident that

$$f(y) = 0 \quad (47)$$

as $F(w) = 0$ (see eqn. 38). The physical meaning of $F'(w_{(\rho')})$ can be the same as that of $\chi^*_{(\rho')}$, so that eqns. 41 and 44 are identical with eqns. 26 and 32 when ε tends to zero. Similarly, eqns. 46 and 47 coincide with eqns. 33 and 35 when $\varepsilon = 0$.

DISCUSSION

In this paper, we have evaluated the variation of the concentration of macromolecules on a column when the slope of the gradient of the activity, y , is very small because, according to earlier numerical calculations, the shape of the chromatogram as a function of y is independent of the slope of the gradient. It should be noted that chromatography carried out using a column with a finite length and a very small gradient slope has to give the same result as that obtained by using a very short column and a finite value of the gradient slope. The chromatographic process in the case of a finite column and a finite slope of the gradient could be expressed as a series of chromatographic steps carried out with an infinite number of columns of infinitesimal length and a constant finite value of the slope of the gradient. We have shown that the shape, as a function of y , of the concentration of macromolecules eluted from a column with an infinitesimal width is independent of the slope of the gradient, *i.e.*, of the value of the proportionality constant between y and V (see Figs. 1-4). Therefore, it is evident that the chromatography is generally independent of the slope of the gradient even in the case of a finite column, which can be considered as the sum of very thin hypothetical sections.

In order to solve the differential equations, eqns. 1a and 16a, we have used the initial conditions given by eqns. 8 and 20, respectively. These conditions do not concern the initial distribution of macromolecules on the crystal surfaces. It is evident that this distribution does not influence the shape of the chromatogram when the slope of the gradient is very small, as the width of the chromatogram is much larger than the length of the column. When the slope of the gradient is large, it could be considered that these initial conditions may give the state where molecules are distributed homogeneously on the column. Actually, we expect that molecules are adsorbed from the top of the column following the affinity to HA (see Part II²). We can show, however, by using numerical calculations, that the shapes of the chromatograms obtained under these two different initial conditions are virtually identical.

APPENDIX I

In Parts II-IV²⁻⁴, chromatograms of several types of mixtures of macromolecules were calculated by dividing the column into a finite number of sections. The chromatograms thus obtained, however, often show fluctuations that are perhaps artifacts due to the mathematical procedure. In this Appendix, we present some results of the analytical calculations of the chromatograms for the same mixtures of molecules as used in Parts II-IV.

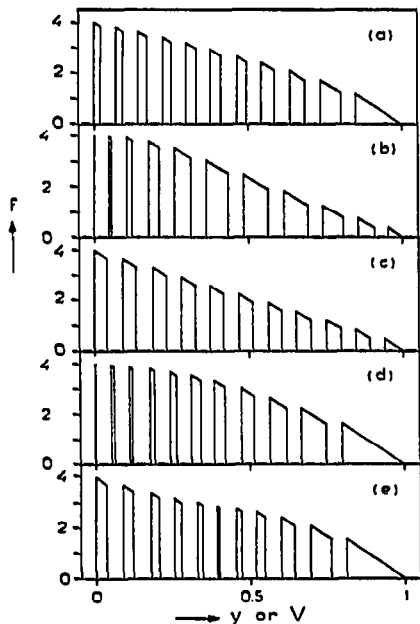


Fig. A1. Illustration of the theoretical chromatograms calculated analytically for mixtures of eleven molecular species shown in Fig. 8 in Part II². It was assumed that

$$\Xi \sqrt{\sum_{\rho'=1}^{11} \chi^*(\rho')} = 0.5.$$

Parts (a)-(e) in Fig. A1 illustrate the results for the mixtures of eleven molecular species shown in parts (a)-(e), respectively, in Fig. 8 in Part II, where we have chosen the value of

$$\Xi \sqrt{\sum_{\rho'=1}^{11} \chi^*(\rho')}$$

as 0.5. Fig. A1b represents the case when the distribution of molecular species in the mixture is Gaussian². Parts (a) and (b) in Fig. A2 show how the shape of the chromatogram in Fig. A1b changes when the column length increases by a factor

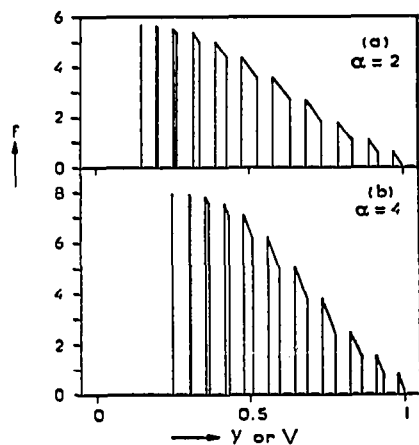


Fig. A2. As Fig. A1b when the column is twice and four times longer, *i.e.*, when $\alpha=2$ and $\alpha=4$, respectively. It was assumed that

$$\Xi \sqrt{\frac{1}{\alpha} \sum_{\rho'=1}^{11} \chi^*_{(\rho')}} = 0.5.$$

For the definition of the parameter α , see text.

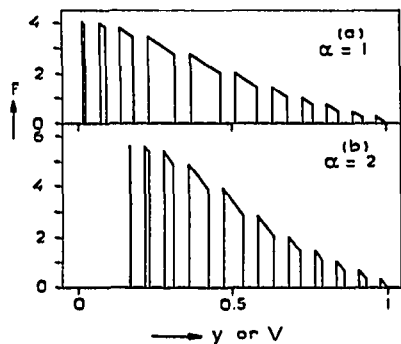


Fig. A3. As Fig. A1 for the mixture shown in Fig. 1b in Part III³. It was assumed that

$$\Xi \sqrt{\frac{1}{\alpha} \sum_{\rho'=1}^{11} \chi^*_{(\rho')}} = 0.5.$$

The column length in part (a) is equal to that in Fig. A1. The column in part (b) is twice as long as that in part (a).

of 2 and 4, respectively. Parts (a) and (b) in Fig. A3 are the chromatograms for the mixture shown in Fig. 1b in Part III³. In part (a), it has been assumed that

$$\Xi \sqrt{\frac{1}{\alpha} \sum_{\rho'=1}^{11} \chi^*_{(\rho')}} = 0.5$$

and in part (b) that the column is twice as long. In each portion of Figs. A1–A3, eleven chromatographic peaks will constitute a right-angled triangle if they are collected so as

to have no intervals among themselves. It should be noted that the widths of the peaks of minor components are generally small and that the height of the peak depends only slightly on the amount of the component. The results of the calculation of chromatograms obtained by dividing the column into a finite number of sections, which correspond to Figs. A1–A3, are shown in Figs. 9–14 in Part II and in Figs. 2 and 3 in Part III, respectively. It can be seen in these figures that the widths of the contributions of minor components are larger than in Figs. A1–A3 and that their heights are much lower. This result is, of course, an artifact of the calculation. However, if one takes into account the fact that there is some axial diffusion of molecules, one might expect that the shapes of the actual chromatograms would be similar to those shown in the figures in Parts II and III, as the smaller is the width of the initial peak, the more important is the effect of diffusion. This effect will increase if the column length increases or if the slope of the gradient increases, because the width of the peak decreases generally when the slope of the gradient increases (see below). It is obvious that the errors that arise from the division of the column into a finite number of sections is more important when the width of the peak is small.

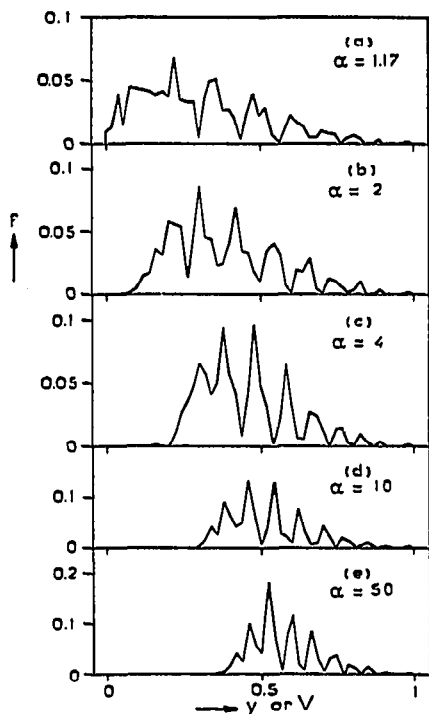


Fig. A4. As Fig. A1 for the mixture including an infinite number of molecular species which is shown in Fig. 1c in Part IV⁴ for several column lengths. It was assumed that

$$\Xi \sqrt{\frac{1}{\alpha}} \int \chi^* d\chi^* = 0.5.$$

In this figure, f is expressed as a relative value.

Fig. A4 illustrates some results for the mixture shown in Fig. 1c in Part IV⁴, where the distribution of molecular species is continuous. For practical purposes, we have divided the value of the activity, y , into elementary steps each of 0.02 unit and estimated the probabilities that molecules appear in these elements. We have also assumed that

$$\Xi \sqrt{\frac{1}{\alpha} \int \chi^* d\chi^*} = 0.5,$$

where α is a constant proportional to the column length. The result obtained by dividing the column into a finite number of hypothetical sections is shown in Fig. 2 in Part IV. Comparing both Fig. A4 in this paper and Fig. 2 in Part IV with Fig. 6 in an earlier paper⁶, which shows the experimental chromatograms, it can be seen that a better agreement with the experiment is obtained by using an analytical calculation if the column is short (compare Fig. A4b with Fig. 6A in ref. 6). If the column is long, however, the calculation made by dividing the column into finite sections gives a better agreement with the experimental results. It is obvious that the shape of the left-hand part of the theoretical chromatogram in each portion of Fig. A4 is not much influenced by the effect of diffusion, as a considerable number of molecules are concentrated in this part, making a "peak" with two or three fluctuations and with a large width. The heights of the other peaks with small widths will decrease significantly by the longitudinal diffusion of molecules when the column is long and a type of chromatograms similar to those obtained experimentally will be produced. It is also probable that the shape of the theoretical chromatogram will be slightly deformed, if one takes into account the fact that the value of x' or the dimensions of molecules are finite.

APPENDIX II

We wish to give a precise definition of the parameter φ or Ξ characterizing mutual interactions of macromolecules on the surface of HA in the general case when both the length and the value of the adsorption energy of the molecules can vary.

The energy of a molecular species ρ' on the crystal surface, $u_{(\rho')} \psi_{(\rho')}(\Theta_3)$, can be written in general as

$$u_{(\rho')} \psi_{(\rho')}(\Theta_3) = u_{(\rho')} - \varphi' b_{(\rho')} \sqrt{\Theta_3} \quad (\text{A1})$$

where φ' is a constant and $b_{(\rho')}$ is the molecular length of species ρ' . We can consider that $2b_{(\rho')} \sqrt{\Theta_3}$ shows the average total length of the parts of the molecule where contact with other molecules is made. Assuming that all molecules have the same diameter, we have

$$b_{(\rho')} \propto x'_{(\rho')} \quad (\text{A2})$$

Therefore, introducing a new constant φ'' and using eqn. 1 in Part V⁵, *i.e.*,

$$w_{(\rho')} = \frac{\xi_{(\rho')}}{\xi_{(1)}} \quad (\text{A3})$$

we can write

$$\begin{aligned}
 \psi_{(\rho')}(\Theta_3) &= 1 - \varphi'' \frac{x'_{(\rho')}}{u_{(\rho')}} \sqrt{\Theta_3} \\
 &= 1 + \varphi'' \frac{x'_{(\rho')}}{x_{(\rho')}\varepsilon_3} \sqrt{\Theta_3} \\
 &= 1 + \frac{\varphi''}{\varepsilon_3} \cdot \frac{1}{\xi_{(\rho')}} \sqrt{\Theta_3} \\
 &= 1 + \frac{\varphi''}{\varepsilon_3 \xi_{(1)}} \cdot \frac{1}{w_{(\rho')}} \sqrt{\Theta_3}
 \end{aligned} \tag{A4}$$

where

$$\frac{\varphi''}{\varepsilon_3 \xi_{(1)}}$$

is a constant. It can be seen that both eqns. 9 and 12 in Part II are included in eqn. A4 if we define φ as

$$\varphi = - \frac{\varphi''}{\varepsilon_3 \xi_{(1)}} \tag{A5}$$

On the other hand, Ξ can always be defined as

$$\Xi = \varphi \sqrt{\Theta_3^S} \tag{A6}$$

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Some of the calculations were performed on the CII 10070 computer of the Faculty of Sciences, University of Paris.

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